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In situ monitoring of chlorpromazine radical intermediate by spectroelectrochemistry

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ABSTRACT

Chlorpromazine has been used widely as an antipsychotic agent and its major metabolic pathways include 7-hydroxylation, N-dealkylation, N-oxidation and S-oxidation. The present work focuses on *in situ* monitoring of the radical intermediate, for clarifying the mechanism diversity in anodic oxidation of chlorpromazine at acidic and physiological pH's. Only in acidic media the colored cation radical was detected by UV–vis spectroelectrochemistry and cyclic voltabsorptometry. The radical was formed at the first anodic peak, followed by a further one-electron oxidation at the second peak to generate the sulfoxide. At physiological pH, the sulfoxide was generated at the first anodic peak through a one-step two-electron oxidation, resulting in much higher current response than at acidic pH's. This work provides an example of a positive and a negative voltabsorptometric peak indicating the formation and conversion of a radical intermediate.

1. Introduction

Up to now over 5000 tricyclic phenothiazine compounds have been obtained and the phenothiazine derivatives have since held a prominent place in pharmacology and biomedicine [1,2]. The field of their applications is constantly growing with discoveries of various properties such as anti-inflammatory [3], antimicrobial [4], antiprionic [5], anticancer and antimalarial effects [6], and interaction with different biological macromolecules [7,8]. Recently, interest in phenothiazine structure has re-emerged for a variety of fascinating features in relation to neurodegenerative disease, spearheaded by the unique redox chemistry of phenothiazine [2].

The most often used phenothiazines are substituted in the positions 2 and 10 [9]. Chlorpromazine (CPZ) (2-chloro-10-(3-dimethylaminopropyl) phenothiazine) is a typical phenothiazine derivative and one of the most widely used antipsychotic drugs. It acts as a potent dopamine receptor antagonist and has function to control excitement, agitation and other psychomotor disturbances in schizophrenic patients and reduces the manic phase of manic-depressive conditions [10].

Metabolism of CPZ is very complex, and the major metabolic pathways include 7-hydroxylation, N-dealkylation, N-oxidation and S-oxidation [11]. The oxidation processes of CPZ and some phenothiazine-based drugs have been studied in different conditions [12–18]. The major metabolite in their biotransformation is the corresponding sulfoxide formed through a cation radical intermediate [12]. Reaction with weak oxidants leads to reversible formation of the cationic radicals without further formation of sulfoxides [13]. The cation radicals itself have been considered as the pharmacologically active form [12], and they are cytotoxic to kill bacteria, plasmids and tumor cells [14]. The cation radicals from oxidation of CPZ can oxidize amino acids [15] and melatonin [16], which may cause oxidative damage. The radical intermediate and final products also exhibit phototoxic effects comparable to or even higher than those of the parent drug [17]. In MeOH and MeCN the singlet oxygen oxidation of CPZ causes side-chain cleavage [18].

Because of the apparent parallelism between chemical oxidation and electrochemical oxidation, the study of electro-oxidation of phenothiazine-based drugs could help in understanding their complex oxidative metabolism mechanisms. In addition, the electrochemical study is of significance for exploring the oxidation nature of the tested substances, independent of oxidants and catalysts used in chemical oxidation. Electrochemical oxidation of CPZ has been investigated, focusing mainly on the electrochemical detection [9,19–23], and less on other aspects such as the generation of cation radicals [12] and the interaction with bilayer lipid membrane [24]. It is commonly believed that the oxidation pathway involves two sequential one-electron steps. The first is reversible and results in the formation of a colored cation radical, whereas the second is irreversible and produces the colorless sulfoxide [21]. Further investigation is needed, because the oxidation

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mechanisms of aromatic amines are found to be complex and highly pH-dependent [25,26].

In the present work, the electro-oxidation of CPZ is investigated comparatively in different pH media. We notice a distinct difference in anodic current responses between CPZ oxidation in physiological and in acidic pH media. The reasons behind this are discussed, based on the *in situ* monitoring of the radical intermediate. A long path-length thin-layer electrochemical cell is used for the experiments of UV–vis spectroelectrochemistry and cyclic voltabsorptometry (CVA) [27,28]. The thin-layer cell allows an opaque graphite working electrode to be used and the light-absorbing species in thin-layer solution to be monitored.

2. Experimental

2.1. Chemicals and solutions

CPZ·HCl (99%+ pure) was purchased from Hubei Kangbaotai (China) and was used as received. Spectrograde graphite powder (320 mesh) and spectrograde paraffin wax (solidification point 62–65 °C) were purchased from Shanghai Chemical Works for preparing the graphite paste electrodes. Graphite sheet was purchased from Tianjin Aidahengsheng Company (China). All other chemicals were of analytical grade.

All solutions were prepared using doubly-distilled water from an all-glass distillatory apparatus. Supporting electrolytes with various pH values were 0.2 M Britton–Robinson (B–R) buffers in water plus 0.5 M KCl. A stock solution with a concentration of 5.0 mM CPZ was prepared in water and then kept at 4 °C in a refrigerator. Before used it was diluted to various convenient concentrations by mixing with the buffer supporting electrolytes. High pure N_2 was used to deaerate the electrolytes.

2.2. Apparatus, cells and electrodes

Cyclic voltammetry (CV) and spectroelectrochemistry were carried out on a CHI660C workstation (Chenhua, Shanghai, China). UV–vis spectroscopic and photometric study was carried out on an UV-2550 spectrophotometer (Shimadzu, Japan) to monitor the soluble reaction products under potentiostatic or potentiodynamic conditions.

A 10-ml volume single-compartment cell was used for the conventional voltammetric measurements. A thin-layer spectroelectrochemical cell was self-made, using a standard quartz photometric cell with 10 mm optical path length as the cell body. The schematic view of the thin-layer cell can be found in the literature [29]. The incident light beam parallels to the working electrode and goes through the thin-layer electrolyte solution (10 mm long, 0.2 mm thick) on the electrode surface.

The three-electrode system was composed of a graphite working electrode, a Ag/AgCl/KCl (saturated) reference electrode and a platinum wire counter electrode. The graphite electrode used in conventional single-compartment cell was a disk solid carbon paste electrode (sCPE) with a smaller geometrical area of 0.049 cm^2. The sCPE was chosen as the working electrode because of its advantages of low background currents, low noise and fast base line stabilization. Its preparation was described previously [30,31]. The electrode used in the thin-layer cell was a quadrate graphite sheet electrode with a larger area of 0.80 cm^2 [29].

2.3. Procedures

Before experiment, the electrochemical cell was washed with water and ethanol successively for 1 min under ultrasonication, and the CPZ solution was deoxygenized with high pure N_2 for about 15 min. The working electrode was polished successively with 800 and 2000 grit emery papers till being a mirror face. Before each run, the working electrode was cleaned and activated by repetitive cyclic scans between −1.2 and 1.5 V in 0.1 M KCl water solution, until only the background current remained. All the CV scans were initiated in the positive direction from an initial potential of 0.0 V. A pre-accumulation step was always performed at the initial potential for 60 s, considering the adsorption of CPZ on graphite electrode.

UV–vis absorption spectra were recorded repeatedly during the thin-layer solution was electrolyzed under double potential step conditions. Blank B–R buffers were used for the spectral baseline correction. Multi-cycle cyclic voltabsorptometry was performed synchronously at double wavelengths to follow the absorbance changes of species in the thin layer solution. All the experiments were carried out at room temperature (22 ± 1 °C).

3. Results and discussion

3.1. Cyclic voltammetry

The oxidation of CPZ was investigated at sCPE by cyclic voltammetry in a pH range of 1.8–7.4. Fig. 1A shows the CV curves measured between 0.0 V and 0.8 V. An anodic peak A1 was observed at about 0.62–0.69 V, along with its cathodic counterpart C1 at about 0.55 V. The peak potentials of both the peaks show little dependence on pH of the buffers, which supports a reaction mechanism without proton transfer. Peak A1 is generally attributed to the formation of CPZ cation radical ($CPZ^{\cdot+}$) *via* one electron oxidation of CPZ, whereas peak C1 is related to the reduction of $CPZ^{\cdot+}$ [19–21]. A very interesting finding is that, at pH 7.4, the peak current

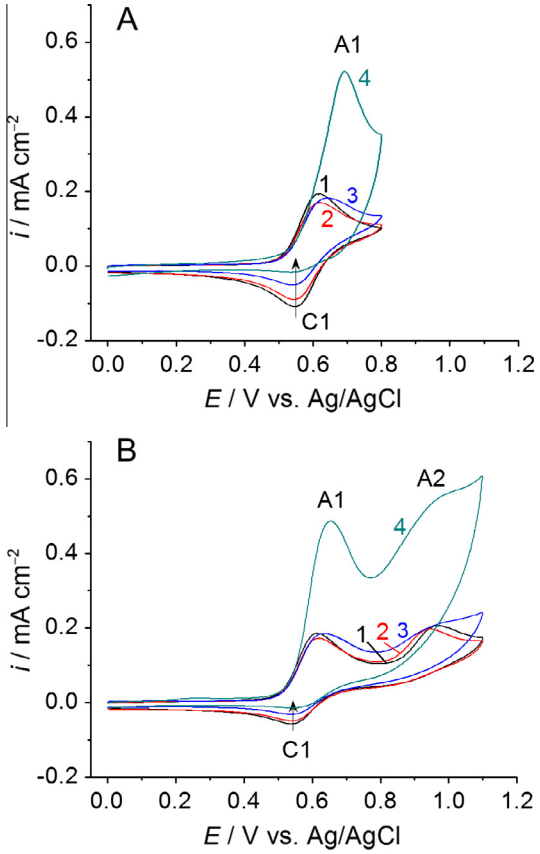

Fig. 1. CV curves of 0.5 mM CPZ in a narrow (A) and wide (B) potential range. pH (1 → 4): 1.8, 3.2, 5.0, 7.4. Scan rate 50 mV s^{-1}.